PB 210 021

Environmental Medicine

RESEARCH STUDY TO DETERMINE THE RANGE OF CARBOXYHEMOGLOBIN IN VARIOUS SEGMENTS OF THE AMERICAN POPULATION

ANNUAL REPORT

OCTOBER 1, 1970 - SEPTEMBER 30, 1971

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Springfield, Va. 22151

PROJECT NO: CRC APRAC CAPM- 8-68
MCOW-ENVM-COHb-71-1

BIBLIOGRAPHIC DATA SHEET	1. Report No. CRC-APRAC-CAPM-8-68-1	2.	3. Recipient's Accession No.
4. Title and Subtitle Research study to Determine the Range of Carboxyhemoglobin in Various Segments of the American Population			5. Report Date September 30, 1971 6.
7. Author(s)			8. Performing Organization Rept. No. MCOW-ENVM COHb 71-1
9. Performing Organization Name and Address The Medical College of Wisconsin 8700 West Wisconsin Avenue Milwaukee, Wisconsin 53226			10. Project/Task/Work Unit No. CRC-APRAC-CAPM-8-68 11. Contract/Grant No.
12. Sponsoring Organization Name and Address Coordinating Research Council, Inc., 30 Rockefeller Plaza, New York, N.Y. 10020 and the Environmental Protection Agency, Research Triangle Park, North Carolina 27711			13. Type of Report & Period Covered Final (10/70-9/71) 14.

15. Supplementary Notes

Releasable to the public June 1, 1972.

16. Abstracts Over the past 12 months, approximately 17,500 blood samples and over 1,000 breath samples were collected from blood donors at 13 major sampling locations throughout the nation. Two of these locations were revisited, so that a total of 15 separate sampling programs have been conducted. Samples collected from the Milwaukee area before the initiation of the COHb Mass Screening Program have been combined with those of the regular Milwaukee study to form a detailed written report. Data from five other cities, New York, Miami, St. Louis, Chicago and Washington, D C. have undergone preliminary computer analysis. A manual analysis of lab tabulated data has also provided up-to-date information on non-smokers for all of the sampling sites and cities thus far visited.

17. Key Words and Document Analysis. 17a. Descriptors

Carbon Monoxide
gas chromatography Carboxyhemoglobin
Carboxyhemoglobin
Cigarettes
Inhalers
Non-inhalers
Bloodstream

17b. Identifiers/Open-Ended Terms

Milwaukee, Wisconsin
New York City, New York
Miami, Florida
St. Louis, Missouri
Chicago, Illinois
Washington, D.C.

17c. COSATI Field/Group 6A, 6F, 6P, 6S

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
Releasable to the public.	UNCLASSIFIED	32
	20. Security Class (This Page) UNCLASSIFIED	22. Price

FORM NTIS-35 (10-70)

USCOMM-DC 40329-P71

RESEARCH STUDY TO DETERMINE THE RANGE OF CARBOXYHEMOGLOBIN IN VARIOUS SEGMENTS OF THE AMERICAN POPULATION

Project No.: CRC APRAC CAPM - 8 - 68

MCOW - ENVM - COHb - 71 - 1

Annual Report
for the period of
October 1, 1970 - September 30, 1971
submitted to the

COORDINATING RESEARCH COUNCIL

and

THE ENVIRONMENTAL PROTECTION AGENCY

by

Department of Environmental Medicine

Medical College of Wisconsin

TABLE OF CONTENTS

I. INTRODUCTION

II. SAMPLING STATISTICS

III. MECHANICS OF THE SAMPLING PROGRAM

- A. Contacts
- B. Collection and Shipping

IV. ANALYSIS OF CO IN BLOOD, BREATH AND AIR

- A. Gas Chromatographic Analysis of CO
- B. Carboxyhemoglobin by Gas Chromatography
- C. CO-Oximeter Model 182

V. STATISTICAL ANALYSIS

- A. Variables Studied
- B. Summary of Milwaukee Data Analysis
- C. COHb Means from Hand Tabulated Data
- D. Relationship Between COHb and Breath CO

VI. YEAR TWO

I. INTRODUCTION

In October of 1970, the blood sampling phase of the research study to determine the range of carboxyhemoglobin (COHb) in various segments of the American population was formally initiated when sample collections began in cooperation with the Milwaukee Blood Bank. In the ensuing weeks, a plan for the efficient sampling, handling and shipping of samples of blood and breath was put into operation. An efficient data gathering and processing system was formed to insure efficient storage and easy retrieval of data for subsequent data analysis.

Over the past 12 months, approximately 17,500 blood samples and over 1,000 breath samples were collected from blood donors at 13 major sampling locations throughout the nation. Two of these locations were re-visited, so that a total of 15 separate sampling programs have been conducted.

The success of the project depends not only upon collection of samples, but upon the ability to analyze those samples, rapidly and with accuracy and precision. Methods used initially for the analysis of CO in blood, breath and air have undergone continual critical review and improvement.

The study design described in the initial protocol has been adhered to with only a few minor exceptions. Two of the sampling locations listed on the protocol, Los Angeles and Salt Lake, have not been visited; however, both of these cities will be worked into Phase II of the sampling program. In general,

the program has been well received and the goals for the first year of sampling adequately attained.

Samples collected from the Milwaukee area before the initiation of the COHb Mass Screening Program have been combined with those of the regular Milwaukee study to form a detailed written report. At this writing, data from five other cities, New York, Miami, St. Louis, Chicago and Washington, D. C. have undergone preliminary computer analysis. A manual analysis of lab tabulated data has also provided up-to-date information on non-smokers for all of the sampling sites and cities thus far visited.

The extent and location of sampling to be conducted over the final year of the survey will be dictated somewhat by the results of the preliminary analysis of segments now completed. Additional samples needed for completing certain population segments for race, sex, age, etc. will require that some cities be re-visited while an effort will also be made to increase the sample size of groups having special occupations (e.g., firemen, taxi cab drivers, housewives), or other distinguishing characteristics (e.g., hospital inpatients, newborn infants, expectant mothers).

II. SAMPLING STATISTICS

Fifteen separate sampling programs were conducted over a 12-month period between October, 1970 and October, 1971. The totals (Table I) represent 187 days of sampling in 13 different cities. Two sampling locations, Milwaukee and Detroit, were re-visited. All of the sampling programs were conducted with blood banks which were affiliated with the National Red Cross Blood Bank Program, and approximately two-thirds of the blood samples were collected on mobile units operated by the collection centers. These mobiles were assigned to sampling sites usually within a radius of 50 miles from the centers, which offered an excellent opportunity to sample donors near their natural home or work environments. Two of the blood banks, New York City and Detroit, operated exclusively from mobile units, while the blood bank at Anchorage was the only center where blood was drawn totally at the blood center.

Through the efforts of contacts established in Anchorage, additional samples have been collected from the Alaskan Eskimo and Indian segments, neither of which are included in the regular Red Cross Blood Collection Program.

The greatest number of samples was collected from Denver (2091) with an average of 87 samples per day. The best daily yield of samples was obtained from New York City, 186 samples per day, and Detroit (148 samples per day). Including an additional 3, 900 blood samples collected at the Milwaukee

County Outpatient Clinic, and at other Milwaukee hospitals prior to October, 1970, a grand total of 21, 314 samples were collected.

Table I contains a summary of the sampling statistics for the first year of the sampling program. In addition to the blood samples reported in Table I a breath samples was collected from every tenth blood donor and an average of 2 ambient air samples were collected in the areas in which the blood samples were being drawn. These samples were also returned to the Environmental Medicine Laboratory where they were analyzed for CO.

TABLE I

Sampling Statistics - COHb Survey Year I

City	Starting Date	Sample Days	Blood Bank - Mobile		Total
Milwaukee #1	10/12/70	12	442	687	1129
Chicago	11/ 4/70	12	271	725	996
Detroit #1	11/20/70	8	0	1184	1184
New York City	12/ 7/70	10	0	1865	1865
D. C.	1/ 4/71	14	119	1494	1613
Miami	1/25/71	12	471	425	896
New Orleans	2/17/71	15	509	42	551
St. Louis	3/ 9/71	10	31	1185	1216
Milwaukee #2	3/20/71		0	720	720
Denver	4/19/71	24	1955	136	2091
Hawaii	6/ 1/71	18	899	301	1200
San Francisco	6/28/71	14	965	407	1372
Seattle	7/19/71	11	587	582	1169
Anchorage	8/ 9/71	18	250	0	250
Detroit #2	9/28/71	9	0	1163	1163
TOTALS		187	6,499	10,916	17,415
			Previous Milwaukee Study		3,900
					21,314

III. MECHANICS OF THE SAMPLING PROGRAM

Our blood sampling program has been well accepted by blood collection centers throughout the country. Our field representatives have reported that, without exception, they have been welcomed warmly and enthusiastically at each blood center thus far visited.

At this writing, only one blood center has been reluctant to participate in our survey on the grounds that they have had several such requests and that these tend to disrupt their operation. Since the center is a key sampling site, attempts to gain their confidence and cooperation will be continued.

We believe that the blood center sampling program is providing data which is valid in representing daily "real life" exposures to carbon monoxide. Sampling at the blood centers is usually prompt (less than 15 minutes), and since the normal half-life of COHb is 4-5 hours, COHb levels in the donors should still be quite representative of CO exposures received in their "natural" environment. This is especially true for samples collected on mobile units since those blood drawings are conducted at locations convenient to the donor's residence or place of employment.

A. Contacts:

Our initial contact with each of the blood collection centers was made by a phone call to the Blood Bank Director or Administrator followed by a detailed written explanation of our COHb Sampling Program. All contacts with the Red Cross Blood Banks

were preceeded by a letter of introduction by Tibor J. Greenwalt, M.D., Medical Director of the Blood Program of the American Red Cross. In some instances, a complete copy of the research protocol was requested for review by a blood center before final approval would be granted.

All personal contacts and field sampling have been conducted by our two traveling research associates, Miss Leigh Platte and Miss Betty Stewart. They have been the key to establishing the efficient field sampling programs, and in gaining the superb support and excellent reputation which we feel this program has enjoyed over the first year.

B. Collection and Shipping:

A trial period during which samples were analyzed at the blood collection center was initiated at the start of the Milwaukee survey. This approach was abandoned when it became evident that an adequate quality control program could not be achieved, and that the efficiency of sample collection would be greatly hampered. Therefore, all blood samples collected in the field have been returned to the Environmental Medicine Laboratory for analysis.

Shipping of blood and breath samples has been exclusively via the United States Mail (primarily Air Mail). Shipping cartons designed to hold up to 30 Vacutainer blood collection tubes, or

12 breath tubes/carton are shipped in padded shipping envelopes. Breakage has been negligible (less than 50 tubes to date), postage costs are about $1.50 per carton, and the average shipping time is 3-4 days.

Blood samples are usually collected with assistance from blood bank personnel. The collection device is a purple-top 5 ml Vacutainer tube containing liquid EDTA as the anticoagulant. This tube has been especially convenient in centers where preliminary blood samples are already drawn with a Vacutainer system. In many centers, however, the donors are not sampled previous to the blood drawing, in which case the sample is drained from the collection tubing following the blood drawing. While the subject is donating his blood he is asked to reply to the questions appearing in the questionnaire shown in Figure 1.

A breath sample is collected from every tenth donor to establish the blood-breath relationship for CO under field sampling conditions. Two ambient CO samples are taken in breath collection tubes at each of the sampling sites at intervals spaced to best represent the ambient concentration of CO during the blood drawing period.

FIGURE 1

MASS CARBOXYHEMOGLOBIN SURVEY

Location:_____ City:_____ Date:_____ Collection Time:_____

Occupation:_____ Compant:_____

Coming From:_____ How Long Ago:_____ minutes

Name:_____ Zip _____

Age:_____ Sex:_____ Height:_____ Weight:_____

RACE	HEALTH	OCCUPATION
1. Caucasian	1. Good	1. Urban In
2. Negro	2. Blood Disease	2. Out
3. Asian	3. Lung Disease	3. Suburban In
4. Mexican	4. Heart Disease	4. Out
5. Am. Indian	5. Kidney Disease	5. Rural In
6. Hawaiian	6. Liver Disease	6. Out
7. Other	7. Other_____	7. Unemployed

SMOKE	QUANTITY/DAY	LAST SMOKED
1. No	1. < ½ pack	1. < 1 hour
2. Cigarettes	2. < 1 pack	2. 1 hour
3. Cigar	3. 1 pack	3. 2-3 hours
4. Pipe	4. 1.5 packs	4. 4-7 hours
	5. 2.0 packs	5. 8-15 hours
	6. 2.5 packs	6. 16-24 hours
	7. 3 or > packs	7. >24 hours

INHALE

1. No
2. Yes

Interviewer

% Sat. Carboxyhemoglobin	ppm Background CO	Date: Analyst:
gm% Hemoglobin	mm Hg Barometric Pressure	CO-Oximeter

IV. ANALYSIS OF CO IN BLOOD, BREATH AND AIR

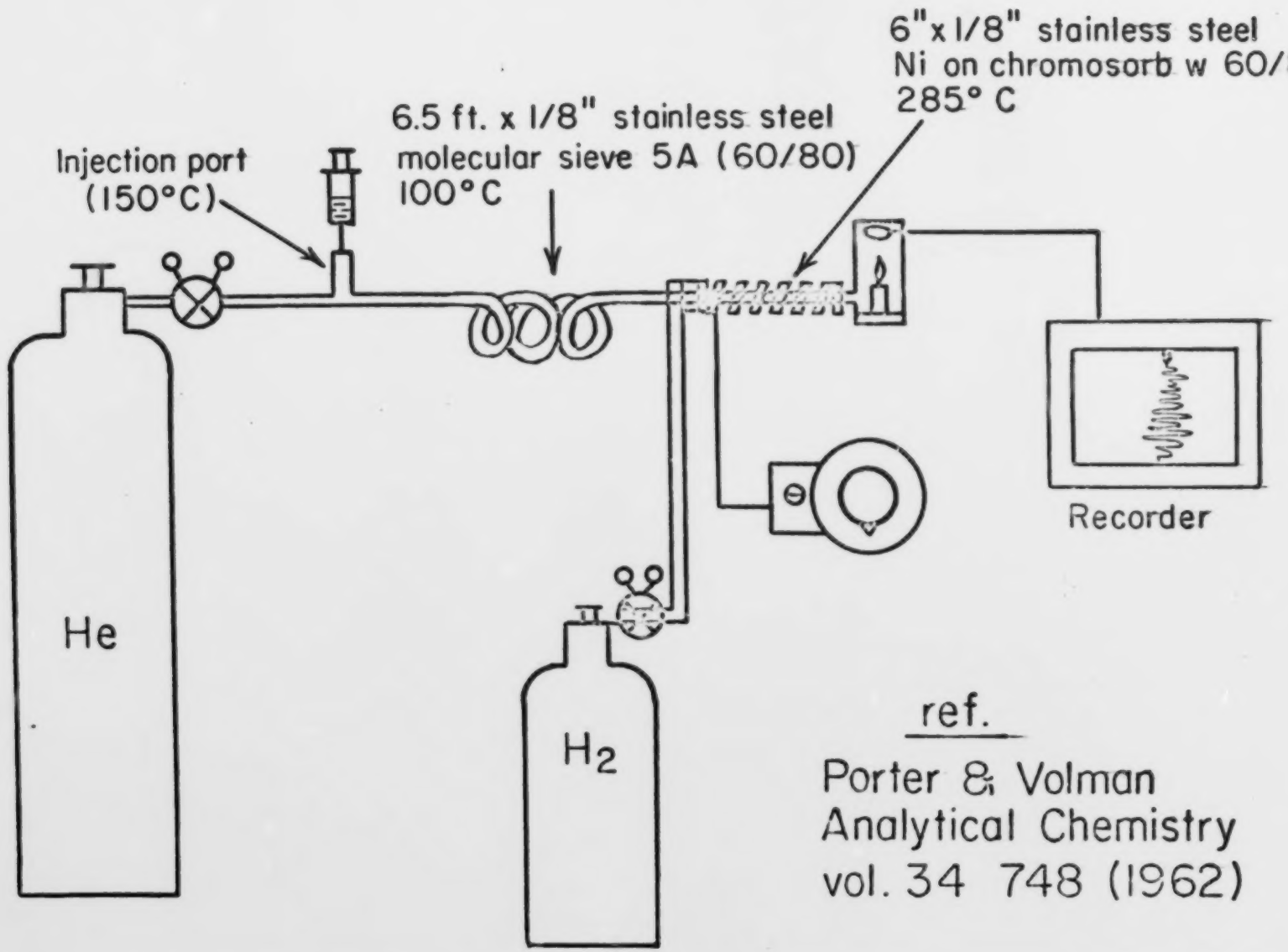
A. Gas Chromatographic Analysis of CO:

The use of gas chromatography (GC) for analyzing low levels of CO in air has lagged somewhat behind infrared techniques. Although it is not difficult to separate CO from other atomospheric gases by gas chromatography, it is difficult to detect CO at ambient or normal breath levels using conventional GC detectors. The GC principle, however, offers the advantage of low sample volume for rapid single sample determinations. The one gas chromatographic detector exquisitively sensitive to CO is the helium ionization system, which was successfully used during the early stages of the mass screening program.

A search of the literature revealed the existence of a more simple and unique system which incorporates the use of a nickel catalyst to convert CO to methane which can then be detected at low concentrations by a conventional hydrogen flame ionization detector (Figure 2). Operating conditions have been optimized to provide an elution time of about 3 minutes. The limit of detection for CO at the conditions described in Figure 3 is about 0.3 ppm.

Standards against which the gas chromatograph are calibrated are prepared by injecting a measured quantity of CO into a Saran bag which contains a known volume of air measured by a

GAS CHROMATOGRAPHIC ANALYSIS of CO by FLAME IONIZATION



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Porter & Volman
Analytical Chemistry
vol. 34 748 (1962)

FIGURE 2

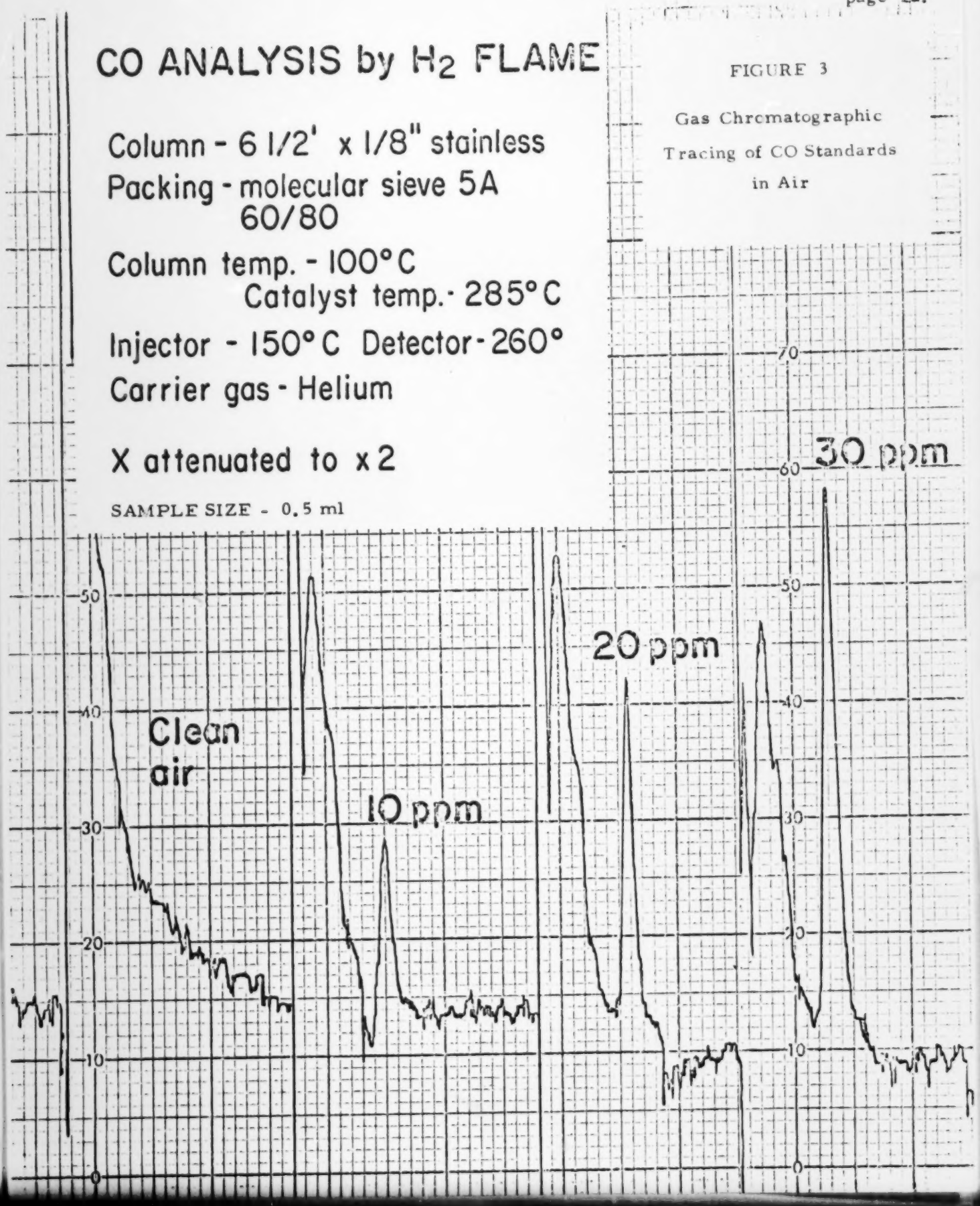


FIGURE 3

Gas Chromatographic Tracing of CO Standards in Air

"Precision" Wet Test Meter. The standards are very stable, since Saran is extremely impermeable to carbon monoxide. To insure the removal of trace quantities of CO, air used in the preparation of standards is passed through a Gas Mask Canister MSA Type N, Model SW, containing a Hopcalite catalyst which oxidizes the Co to CO_2.

B. COHb by Gas Chromatography:

The gas chromatographic system used for the analysis of breath and ambient air samples is also useful for the determination of COHb. The method involves the release of CO from its hemoglobin bound state with a small quantity of concentrated sulfuric acid. The reaction is carried out in a tightly closed reaction vessel of known volume so that the gaseous contents of the vessel containing the CO released can be analyzed for CO content and the percent COHb then calculated.

C. The IL CO-Oximeter Model 182:

Instrumentation Laboratories', Inc. CO-Oximeter Model 182 provides one with a rapid and precise means of analyzing for COHb, O_2Hb, and Hb in one simple operation, with a cycle time of approximately 1 minute/sample. With the CO-Oximeter it is possible to analyze up to about 200 field samples in one eight-hour work shift

(not including the additional samples which must be run to maintain quality control in the operation).

One of the disadvantages in using this instrument, however, is that it must be calibrated against a known standard of human blood. The gas chromatographic method for determining CO in blood provides the analyst with an absolute method of analysis needed for the standardization of blood samples which can then be used to calibrate the CO-Oximeter 182. Quality control is maintained by: (a) daily calibration of the instrument against "carry-over" blood, standardized by the independent gas chromatographic method; (b) daily calibration against blood standards which have been specially prepared, standardized, and stored at freezer temperature; and (c) comparison of readings for every tenth sample on two IL CO-Oximeter instruments which are always calibrated and in operation to insure perfect agreement.

Studies conducted to determine the reproducibility of values on the CO-Oximeter show that values for COHb are repeatable within a 0.2 unit spread ($\pm$ 0.1% COHb) when the same blood sample is undergoing repeated analysis at one sitting. Readings taken over a period of several days are not as precise, e.g., ten readings taken over a period of sixteen days using the same machine produced a spread of 0.6% Hb and 0.4% COHb. Actual mean and standard deviations were:

Hgb%	15.2$\pm$0.19
COHb%	1.2$\pm$0.13

V. STATISTICAL ANALYSIS OF DATA

A. Variables Studied:

A total statistical analysis for the cities sampled during the first year of the COHb Study is being prepared by A. A. Rimm, Ph.D. and his assistants at the Department of Biostatistics of the Medical College of Wisconsin. At this writing, a detailed statistical report has been prepared by the Department of Biostatistics covering data for the Milwaukee area. Preliminary computer analysis has also been prepared from data obtained from Miami, Chicago, New York, St. Louis and Washington, D.C. Data from other cities are in various stages of being processed. Following is a list and a description of the variables studied in the preliminary analysis of the data:

Variable	Description
Age, height, weight, sex	
Race	White, Black, Asian, Mexican, Am. Indian, Hawaiian, other
Health	Healthy: diseases; blood, lung, heart, kidney, liver, other
Occupation	23 major categories
Occupation Location	Urban In, Urban Out, etc. (see questionnaire)
Where Coming From	Home, work, other
Items Smoked	None, cigarettes, cigar, pipe, combination

Variable	Description
Inhale	Yes/No
Packs/Day	0 5 packs increments
Hours Ago Last Smoked	7 levels (see questionnaire)
Background CO Level	To the nearest 1 ppm
Barometric Pressure	During Sampling
Carboxyhemoglobin Level	To the nearest 0.1%
Hemoglobin	To the nearest 0.1 gm%
Sample Time	Hour of Day

Other variables, such as meteorological conditions and air pollution levels, will be worked into the study whenever information is available. Breath-blood data is being analyzed separately.

Some generalizations drawn from the computation lab's analysis of COHb data follow:

1. Data Description:

The distribution of COHb levels is clearly not "normal". Rather, it is skewed to the right due to the great impact of tobacco smoking on COHb levels By comparison, the hemoglobin levels do produce a normal curve. This would suggest at this point at least that hemoglobin levels are not associated with COHb smoking interrelationships.

2. Factors Associated with COHb Levels:

Assuming that the study constitutes a random sampling

of the population, some of the factors associated with COHb levels can be studied without fear of bias. That assumption is made for the following remarks.

All data collected thus far shows quite clearly that cigarette smokers have a significantly higher COHb level than non-smokers. The following data from the Milwaukee study also shows that the standard deviation for COHb in non-smokers is about 1/3 that for smokers. This is expected because of the variability of smoking habits.

	Non-Smokers	Smokers
N	2798	1620
$\bar{X}$	1.33	4.47
σ	0.85	2.52
SEM	0.02	0.06

The trend of COHb levels with increasing cigarette consumption for the Milwaukee Study is shown in Figure 4. This figure illustrates the great influence of cigarette smoking while it also suggests that a "COHb saturation" level does exist. For Milwaukee smokers, this level appears to be near 1.5 packs/day.

As might be expected from each cigarette level, inhalers had a significantly higher COHb than non-inhalers. Time from the last cigarette smoked to the drawing of the sample is shown to be related in Figure 5. The precipitous

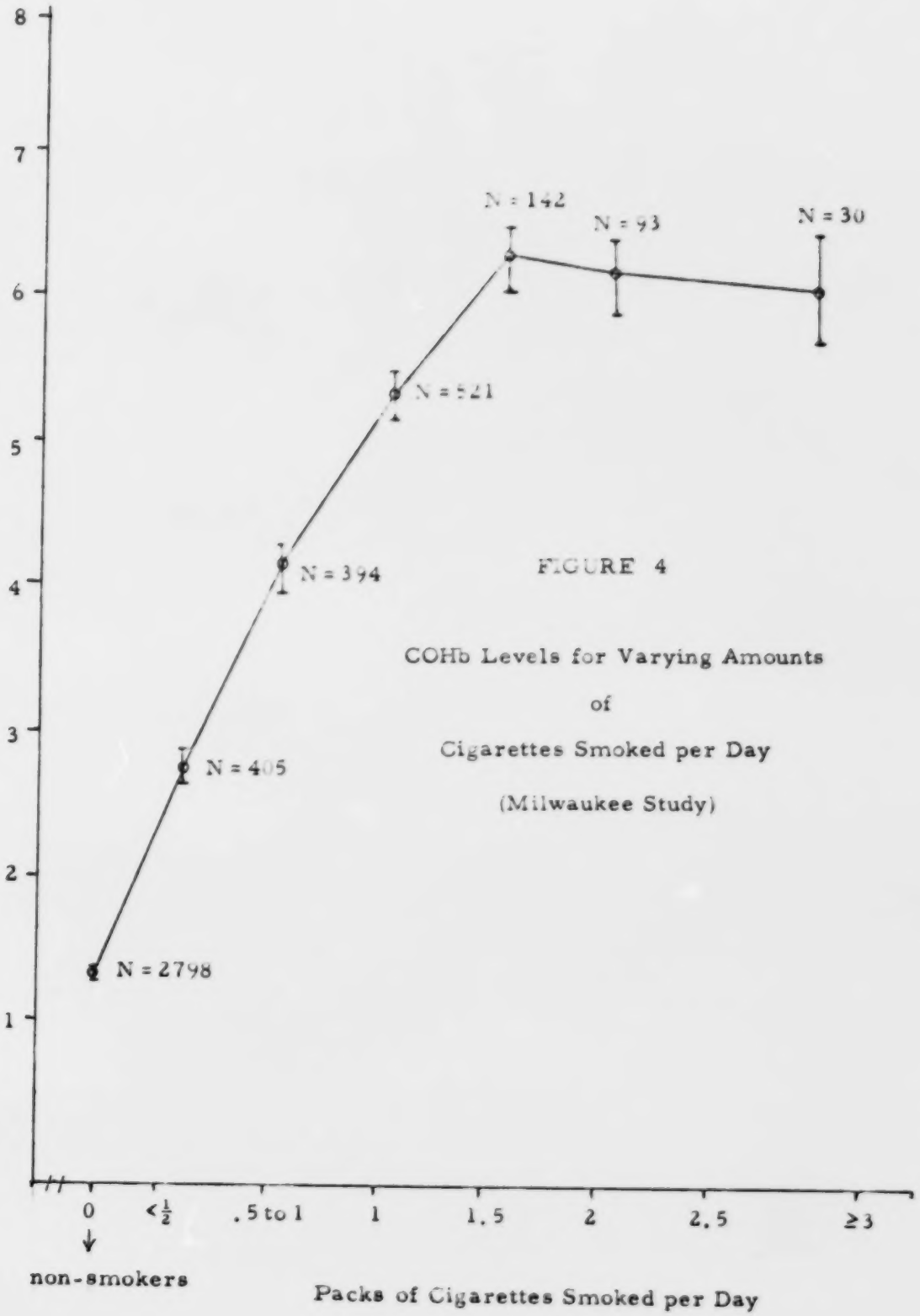


FIGURE 4

COHb Levels for Varying Amounts

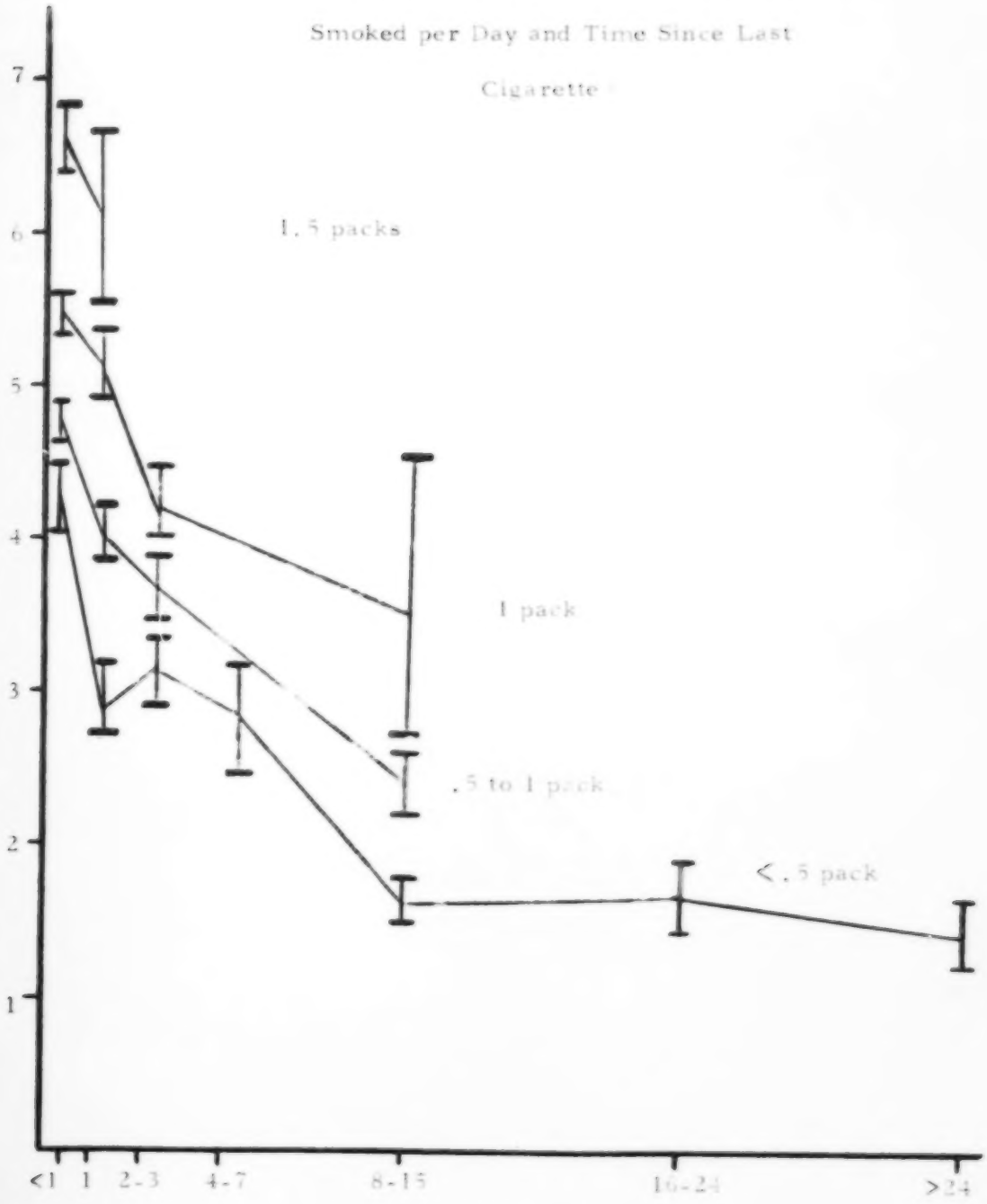
of

Cigarettes Smoked per Day

(Milwaukee Study)

FIGURE 3

COHb Levels for Varying Amounts of Cigarettes Smoked per Day and Time Since Last Cigarette*



*Groups of fewer than 15 people were not plotted.

drop in the more elevated COHb levels followed by a more gradual decline in the slope is characteristic of the half-life decay phenomenon exhibited by COHb circulating in the blood stream.

Mean COHb levels of cigar and pipe smokers is significantly lower than cigarette smokers. However, some of the highest COHb levels measured (up to 20% COHb) have been on cigar smokers who inhale. This presumably is due to the higher concentration of CO in cigar smoke.

B. Summary of Milwaukee Analysis:

Some of the relationships established were unmistakable and predictable, while others at this point are merely suggestive. Following are some of the basic conclusions derived from the study of the Milwaukee data:

1. Of all the variables studied, cigarette smoking has the greatest effect on COHb level;
2. Because smoking has such an overwhelming effect on COHb levels, most relationships can only be studied using samples from non-smokers. Based on non-smokers:
   a. COHb levels as compared to age, weight and residence location show no significant trends;
   b. Significant differences in COHb levels were found for certain groups of sex, race and health status;

however, these differences were small and comments will be reserved until a broader and more complete sampling is obtained;

c. Hemoglobin levels for males were significantly higher than for females; however, no relationship between hemoglobin values and smoking is suggested.

3. In the non-smoking group, the strongest variables which result from the multivariate analysis of data were time of day, weight, and hemoglobin. However, these only explain 1% of the variability of COHb levels, while for cigarette smokers the number of packs smoked per day accounts for over 30% of the variability of COHb levels.

4. Repeatability of measurement of COHb on the same subject (this resulted from repeat samplings conducted in the Milwaukee survey) showed that even though there was a significant difference between normal non-smokers, most of the variation is associated with differences between measurements on different days. In other words, the day-to-day differences on one person are larger than the differences in measurements between people.

C. <u>COHb Means from Hand Tabulated Data:</u>

While the results of questionnaire data and analytical results are being processed for computer analysis, mean carboxyhemoglobin data for non-smokers have been tabulated manually for each sampling site

within each city, and a combined weighted mean for each city is presented in Table II.

Standard "t" and F tests were used for comparing the COHb mean and Standard Deviations for the 15 sampling locations. In Table II the cities are listed in ascending order, with respect to their mean COHb levels. Pairs, or groups underlined are not significantly different from each other at the 95% Confidence Level. However, each of these groups or individual mean values (not underlined) varies significantly from all other values in the table for either the "t" or F test.

Mean carboxyhemoglobin values usually varied as widely between individual sampling sites or groups within each city as they did between cities. This was because samples from people in downtown locations and airports had consistently higher COHb levels than housewives and rural inhabitants. COHb levels are also affected by occupation. For example, the higher mean COHb level in the second Milwaukee study is due to high values from a large sampling of Milwaukee firemen. Taxi cab drivers and traffic policemen similarly often have COHb's over 2%. This is an example of the interdependency of variables which will need to be defined by expert analysis of the massive quantities of data generated by this study.

D. Relationship Between COHb and Breath CO:

Breath analysis is gaining in popularity and usefulness as a method of assessing the total body burden of a volatile substance which appears

TABLE II

Standard "t" and F Test Comparing COHb Mean & Standard Deviation

for 14 Cities

	Milwaukee #1	Detroit #2	Miami	D. C.	St. Louis	New York	Hawaii	Milwaukee #2	Seattle	Detroit #1	San Francisco	New Orleans	Alaska	Chicago	Denver
N	603	596	282	839	675	931	517	225	600	615	664	161	105	417	577
$\bar{x}$ (%COHb)	1.30	1.34	1.38	1.39	1.40	1.43	1.45	1.51	1.56	1.58	1.65	1.72	1.75	1.88	1.90
S.D.	0.60	0.52	0.69	0.61	0.45	0.61	0.56	0.80	0.58	0.69	0.57	0.59	0.68	0.61	0.74

The COHb Mean or Standard Deviation for all the cities above each line are not significantly different at the 95% Confidence Level.

in the bloodstream. Furthermore, if one can analyze the breath for the volatile substance at various intervals following an exposure to a known concentration of that substance, a breath decay curve can be established. One then has an index against which he can make comparisons and estimate with reasonable accuracy the time weighted average exposure to the substance.

The concentration of CO in the breath is truly representative of COHb levels in the blood, and COHb levels therefore can be equated to an exposure level for any estimated exposure time to arrive at a "time weighted average exposure concentration".

Variations in the collection technique and in the quality of the device used for the collection allow for broad variations in breath analysis results. Therefore, CO in its more permanent COHb form is still a more reliable, though less accessible, means of determining CO content of the blood.

The equation derived to best describe the relationship between CO in the breath and % COHb of the blood is:

$$Y = 4X - 1.9$$

where:

Y = CO (ppm) in the alveolar breath

X = percent COHb in the blood

This equation was derived from 341 data pairs in which COHb was analyzed by CO-Oximeter and the corresponding breath CO concentrations were analyzed by either gas chromatography or by

infrared spectrophotometry. These 341 data points are based on samples collected from volunteers of human exposure experiments who were trained and considered adept at collecting samples of their own breath. The samples were collected in glass breath tubes identical to those used in our present survey.

Since the initiation of the Mass Carboxyhemoglobin Screening Program, an additional 1,000 breath samples have been collected in the field, along with corresponding blood samples. Figure 6 describes and compares the two groups of data just discussed. The curve for field collected samples represents all samples, i.e., no attempt was made to screen out obvious leakers and no allowance was made for loss of CO from the tubes in transit. (Although laboratory investigations show this loss is almost negligible.)

As expected, breath samples corresponding to any given blood COHb level produce lower CO levels on the average for field collected samples. Comparison of correlation coefficients (also listed in Figure 6) indicates the much broader scatter of data obtained by field sampling. However, regardless of this scatter, the field data curve was well defined with less than 400 samples (as described by the curve presented at our first Review Meeting in March, 1971).

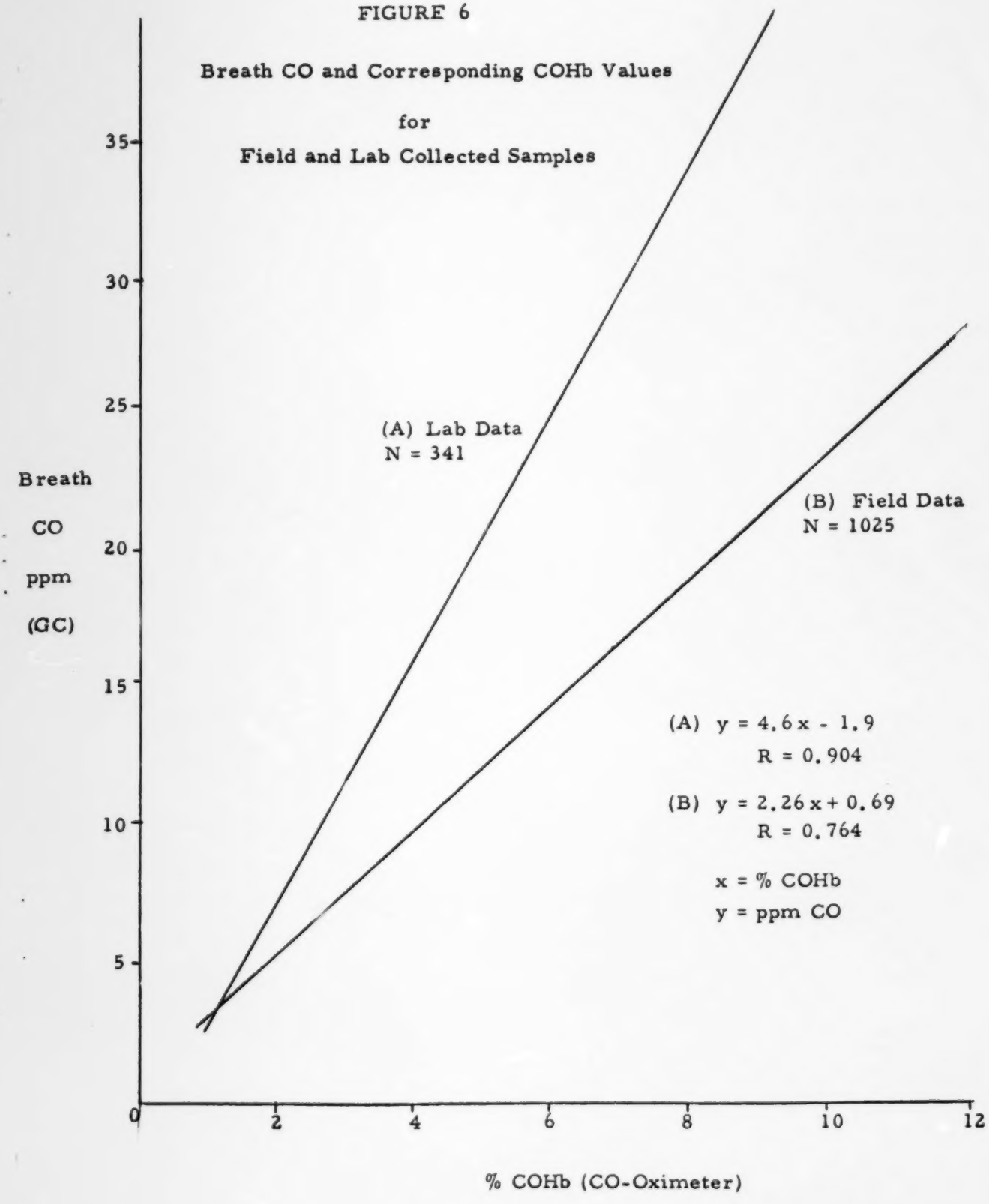
In summary, although it appears to be extremely risky to attempt an assessment of CO exposure on the basis of individual samples, it seems reasonable to conclude that breath analysis on a mass sampling basis might be a useful and reliable index of exposure to CO.

FIGURE 6

Breath CO and Corresponding COHb Values

for

Field and Lab Collected Samples



VI. YEAR TWO

In Year Two the emphasis will be for continued effort in fulfilling the following requirements:

1. Conduct the sampling programs in Los Angeles and Salt Lake City;
2. Fill the required "sample cells" for sex, race, age, etc., as described in the Mass Screening Protocol for as many of the cities already visited as time will allow;
3. Attempt to sample as many "special groups" as possible (including occupational groups, newborn infants, expectant mothers, hospital inpatients, etc.);
4. Obtain a truly rural sampling population;
5. Complete laboratory investigations as outlined in the Protocol to establish:
   a. COHb levels arising solely from endogenous production of CO; and
   b. Immediate effects of smoking upon COHb level
6. Continue and finalize the computer data analysis relating COHb and the variables studied.